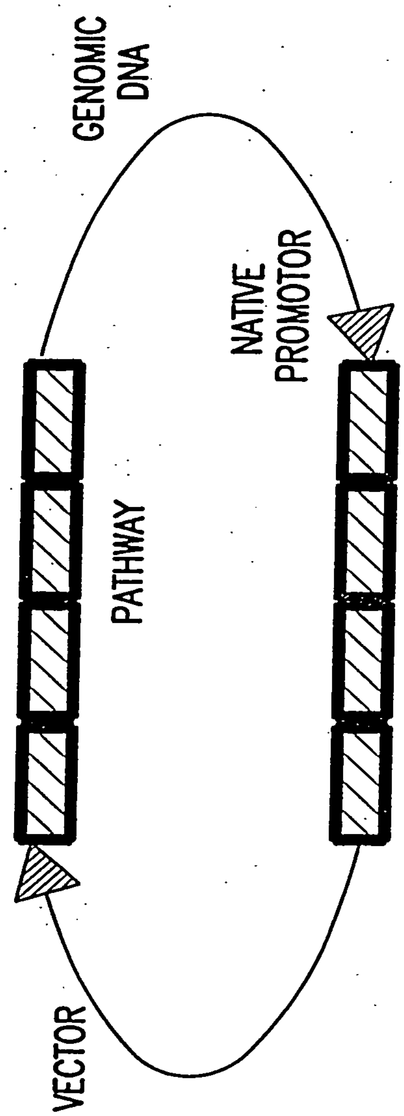


FIG.1

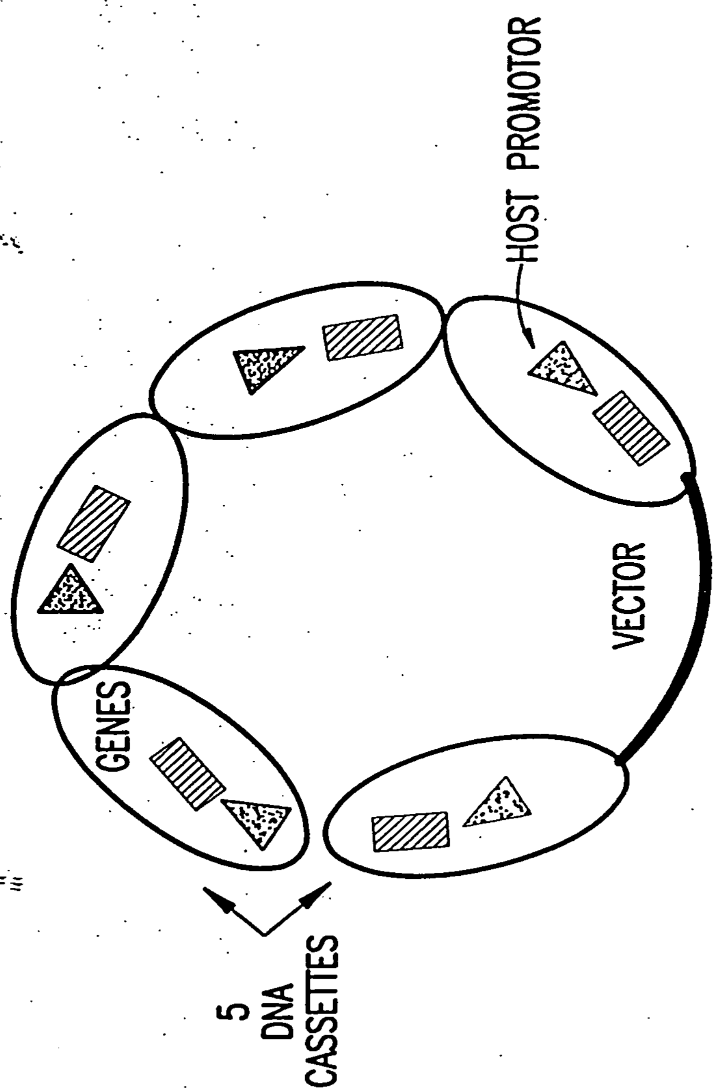


FIG.2

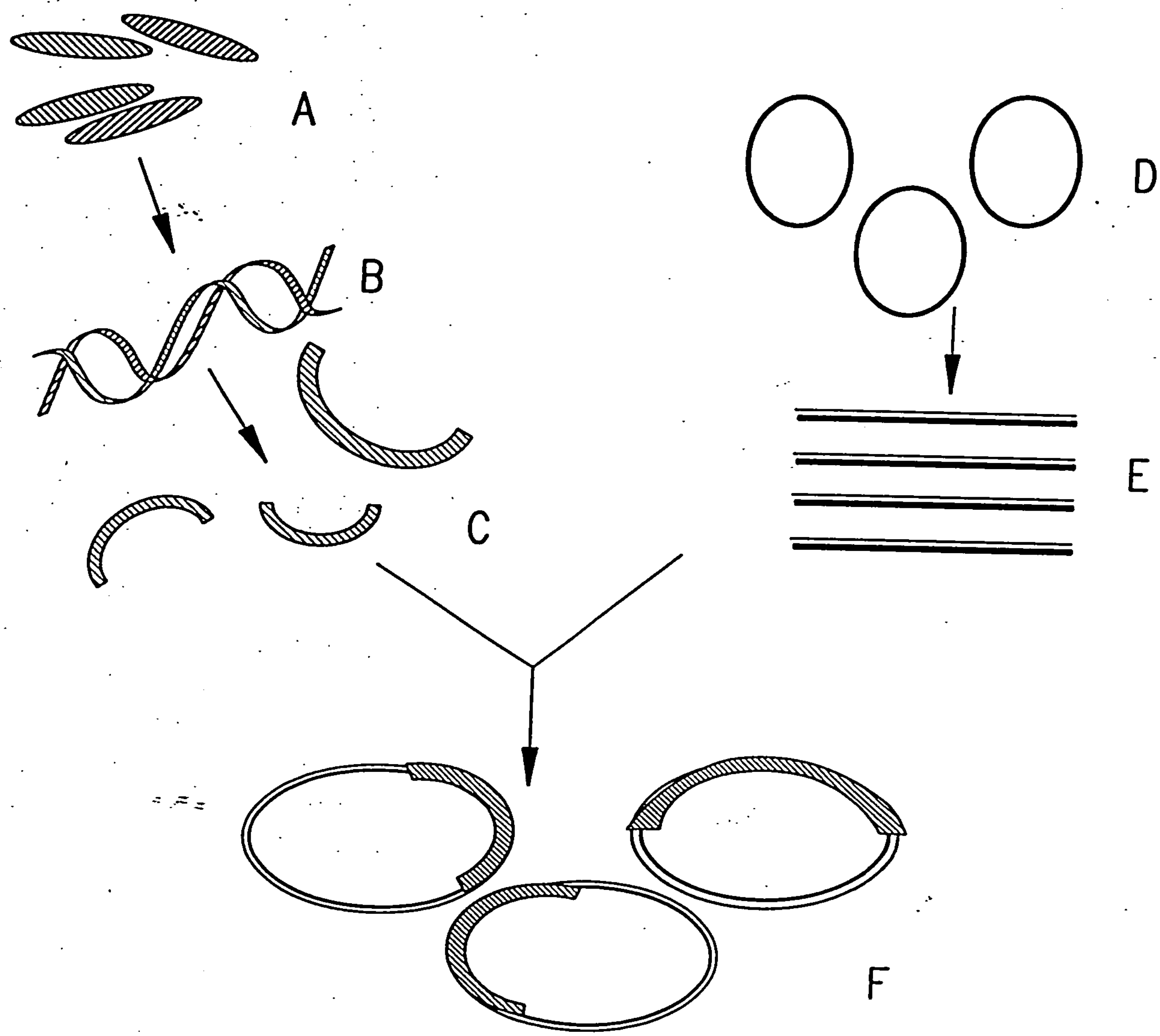


FIG.3

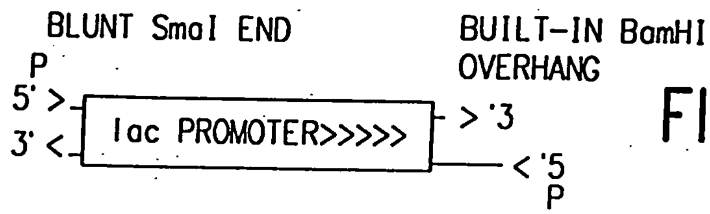


FIG.4A

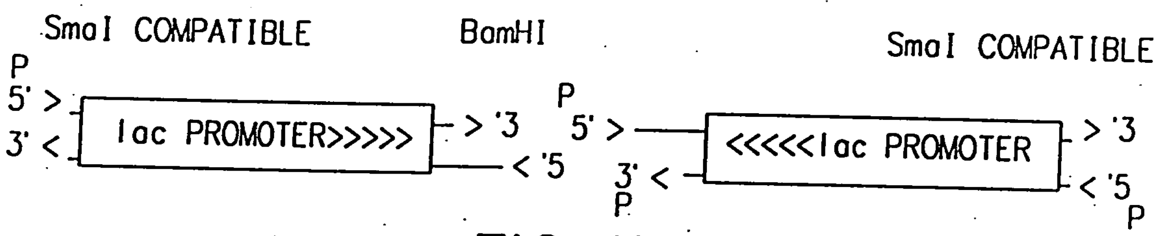


FIG.4B

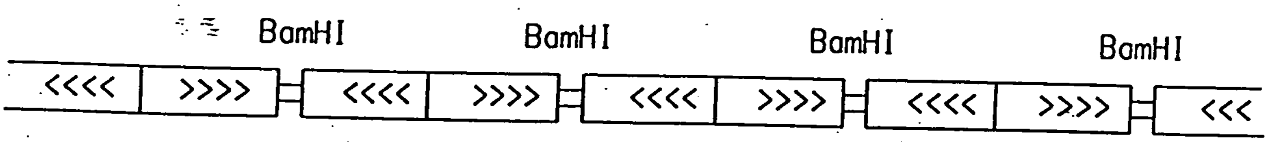


FIG.4C

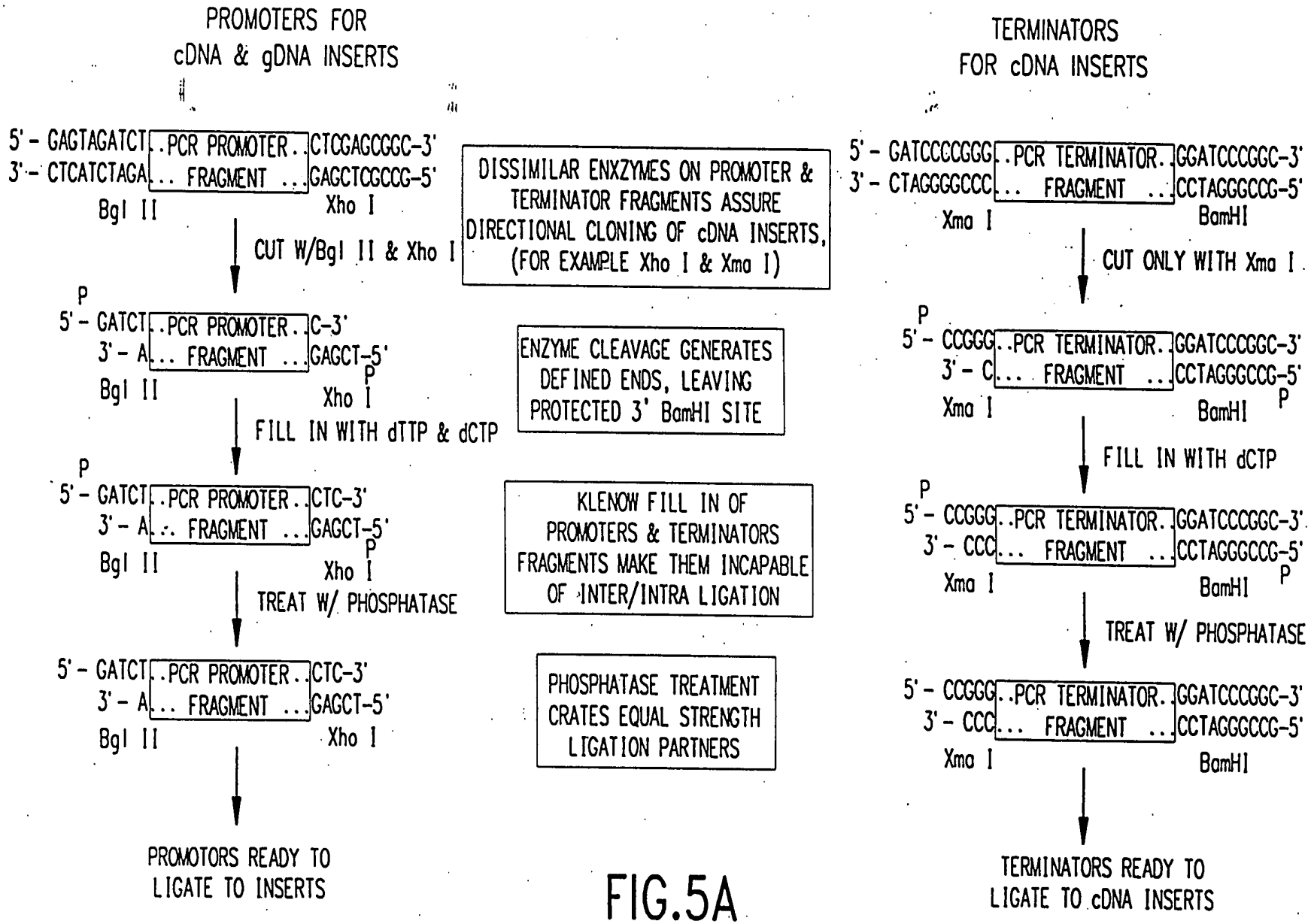

PROMOTERS FOR
cDNA & gDNA INSERTS
5' - GAGTAGATCT .PCR PROMOTER. CTCGAGCGGC-3'
3' - CTCATCTAGA .. FRAGMENT ... GAGCTCGCCG-5'
Bgl II
Xho I
CUT W/Bgl II & Xho I
P
5' - GATCT .PCR PROMOTER. C-3'
3' - A .. FRAGMENT ... GAGCT-5'
Bgl II
P
Xho I
FILL IN WITH dTTP & dCTP
P
5' - GATCT .PCR PROMOTER. CTC-3'
3' - A .. FRAGMENT ... GAGCT-5'
Bgl II
P
Xho I
TREAT W/ PHOSPHATASE
5' - GATCT .PCR PROMOTER. CTC-3'
3' - A .. FRAGMENT ... GAGCT-5'
Bgl II
Xho I
PROMOTORS READY TO
LIGATE TO INSERTS
DISSIMILAR ENXZYMES ON PROMOTER & TERMINATOR FRAGMENTS ASSURE DIRECTIONAL CLONING OF cDNA INSERTS, (FOR EXAMPLE Xho I & Xma I)
ENZYME CLEAVAGE GENERATES DEFINED ENDS, LEAVING PROTECTED 3' BamHI SITE
KLENOW FILL IN OF PROMOTERS & TERMINATORS FRAGMENTS MAKE THEM INCAPABLE OF INTER/INTRA LIGATION
PHOSPHATASE TREATMENT CRATES EQUAL STRENGTH LIGATION PARTNERS
TERMINATORS
FOR cDNA INSERTS
5' - GATCCCCGGG .PCR TERMINATOR. GGATCCCGGC-3'
3' - CTAGGGGCCC .. FRAGMENT .. CCTAGGGCCG-5'
Xma I
BamHI
CUT ONLY WITH Xma I
P
5' - CCGGG .PCR TERMINATOR. GGATCCCGGC-3'
3' - C .. FRAGMENT .. CCTAGGGCCG-5'
Xma I
BamHI
P
FILL IN WITH dCTP
P
5' - CCGGG .PCR TERMINATOR. GGATCCCGGC-3'
3' - CCC .. FRAGMENT .. CCTAGGGCCG-5'
Xma I
BamHI
P
TREAT W/ PHOSPHATASE
5' - CCGGG .PCR TERMINATOR. GGATCCCGGC-3'
3' - CCC .. FRAGMENT .. CCTAGGGCCG-5'
Xma I
BamHI
TERMINATORS READY TO
LIGATE TO cDNA INSERTS

FIG.5A

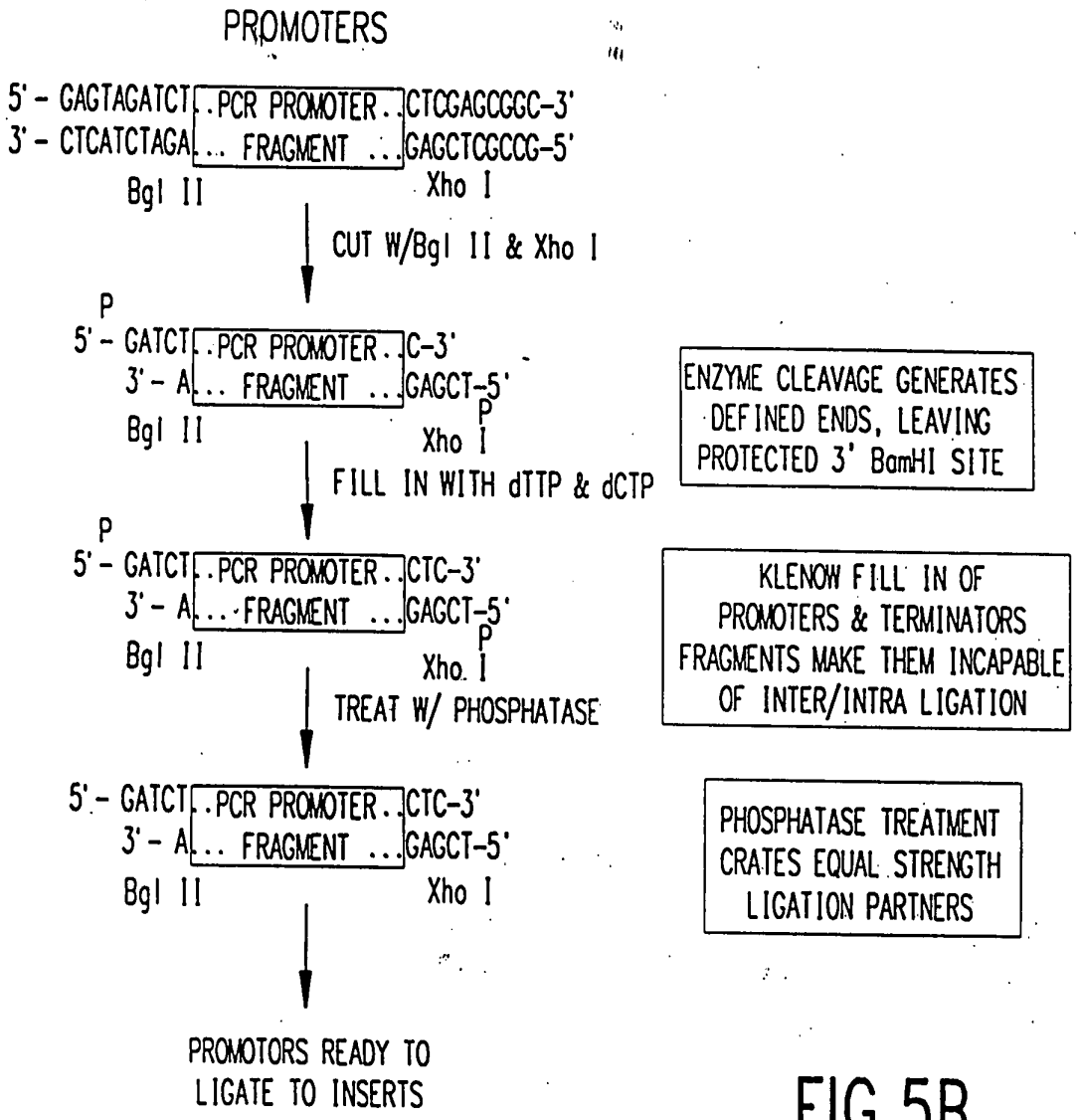

PROMOTERS
5' - GAGTAGATCT .PCR PROMOTER. CTCGAGCGGC-3'
3' - CTCATCTAGA ... FRAGMENT ... GAGCTCGCCG-5'
Bgl II
Xho I
CUT W/Bgl II & Xho I
P
5' - GATCT .PCR PROMOTER. C-3'
3' - A ... FRAGMENT .. GAGCT-5'
P
Bgl II
Xho I
FILL IN WITH dTTP & dCTP
P
5' - GATCT .PCR PROMOTER. CTC-3'
3' - A ... FRAGMENT ... GAGCT-5'
P
Bgl II
Xho I
TREAT W/ PHOSPHATASE
5' - GATCT .PCR PROMOTER. CTC-3'
3' - A ... FRAGMENT ... GAGCT-5'
Bgl II
Xho I
PROMOTORS READY TO LIGATE TO INSERTS
ENZYME CLEAVAGE GENERATES DEFINED ENDS, LEAVING PROTECTED 3' BamHI SITE
KLENOW FILL IN OF PROMOTERS & TERMINATORS FRAGMENTS MAKE THEM INCAPABLE OF INTER/INTRA LIGATION
PHOSPHATASE TREATMENT CRATES EQUAL STRENGTH LIGATION PARTNERS
TERMINATORS
5' - CGGCCTCGAG .PCR TERMINATOR. GGATCCCGGC-3'
3' - GCCGGAGCTC ... FRAGMENT .. CCTAGGGCCG-5'
Xho I
BamHI
CUT ONLY WITH Xho I
P
5' - TCGAG .PCR TERMINATOR. GGATCCCGGC-3'
3' - C ... FRAGMENT .. CCTAGGGCCG-5'
P
Xho I
BamHI
FILL IN WITH dTTP & dCTP
P
5' - TCGAG .PCR TERMINATOR. GGATCCCGGC-3'
3' - CTC ... FRAGMENT .. CCTAGGGCCG-5'
P
Xho I
BamHI
TREAT W/ PHOSPHATASE
5' - TCGAG .PCR TERMINATOR. GGATCCCGGC-3'
3' - CTC ... FRAGMENT .. CCTAGGGCCG-5'
Xho I
BamHI
TERMINATORS READY TO LIGATE TO INSERTS

FIG.5B

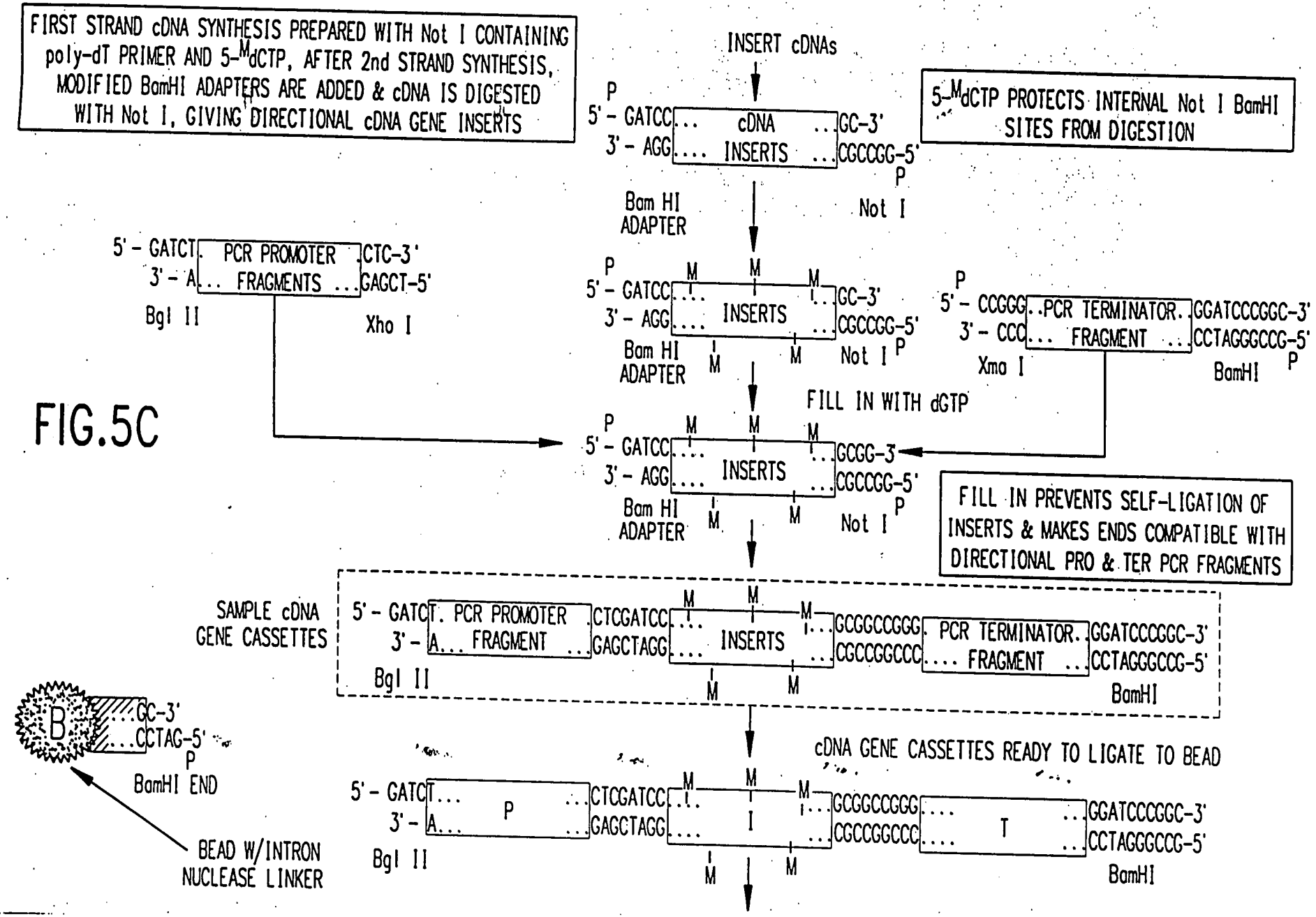

FIRST STRAND cDNA SYNTHESIS PREPARED WITH Not I CONTAINING poly-dT PRIMER AND 5-MdCTP, AFTER 2nd STRAND SYNTHESIS, MODIFIED BamHI ADAPTERS ARE ADDED & cDNA IS DIGESTED WITH Not I, GIVING DIRECTIONAL cDNA GENE INSERTS
INSERT cDNAs
5-MdCTP PROTECTS INTERNAL Not I BamHI SITES FROM DIGESTION
5' - GATCC .. cDNA .. GC-3'
3' - AGG ... INSERTS .. CGCCGG-5'
Bam HI ADAPTER
Not I
5' - GATCT PCR PROMOTER CTC-3'
3' - A ... FRAGMENTS ... GAGCT-5'
Bgl II
Xho I
5' - CCGGG .PCR TERMINATOR. GGATCCCGGC-3'
3' - CCC ... FRAGMENT .. CCTAGGGCCG-5'
Xma I
BamHI
FILL IN WITH dGTP
FILL IN PREVENTS SELF-LIGATION OF INSERTS & MAKES ENDS COMPATIBLE WITH DIRECTIONAL PRO & TER PCR FRAGMENTS
SAMPLE cDNA GENE CASSETTES
cDNA GENE CASSETTES READY TO LIGATE TO BEAD
B
GC-3'
CCTAG-5'
BamHI END
BEAD W/INTRON NUCLEASE LINKER

FIG.5C

FIG.5D

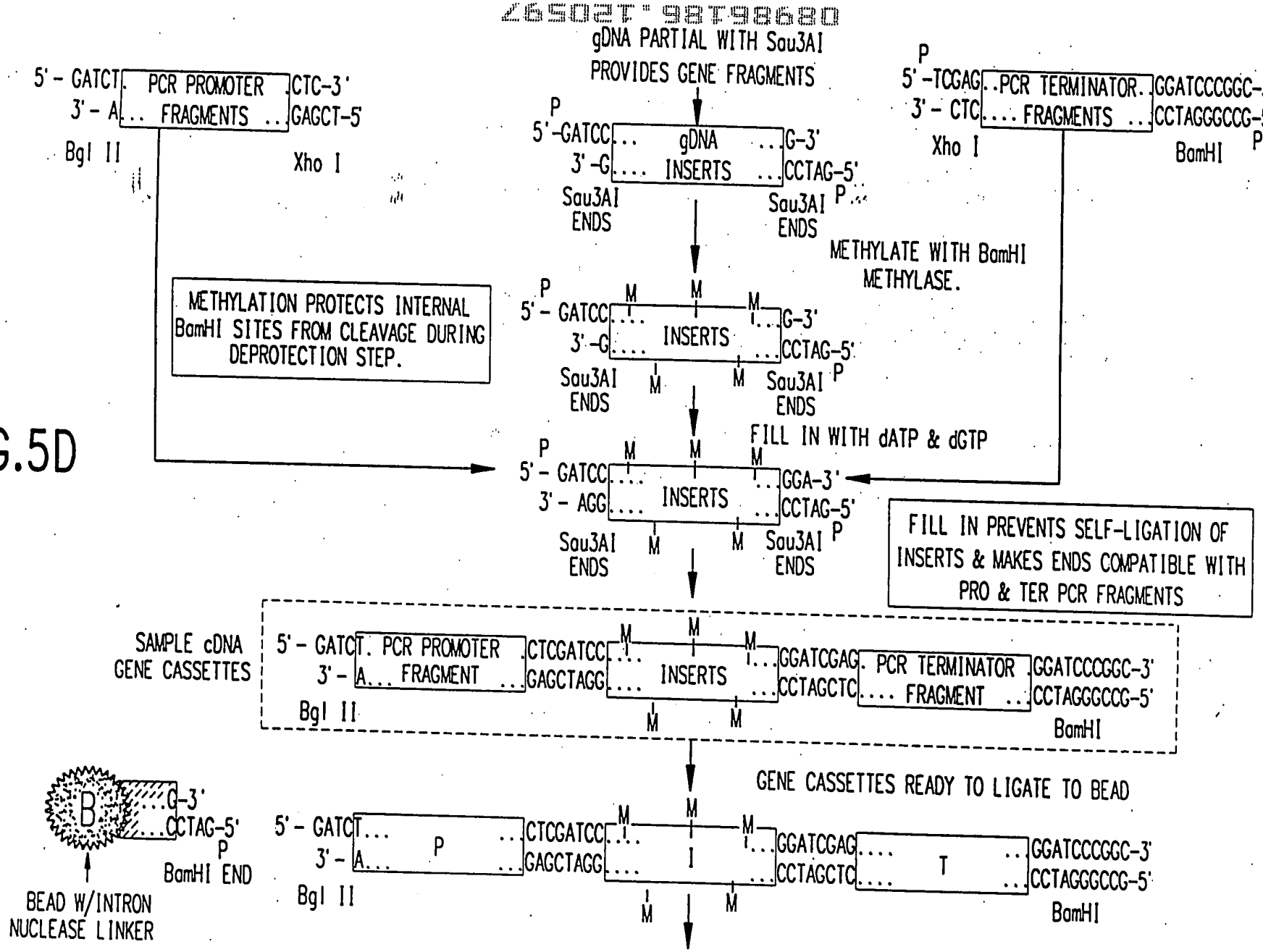
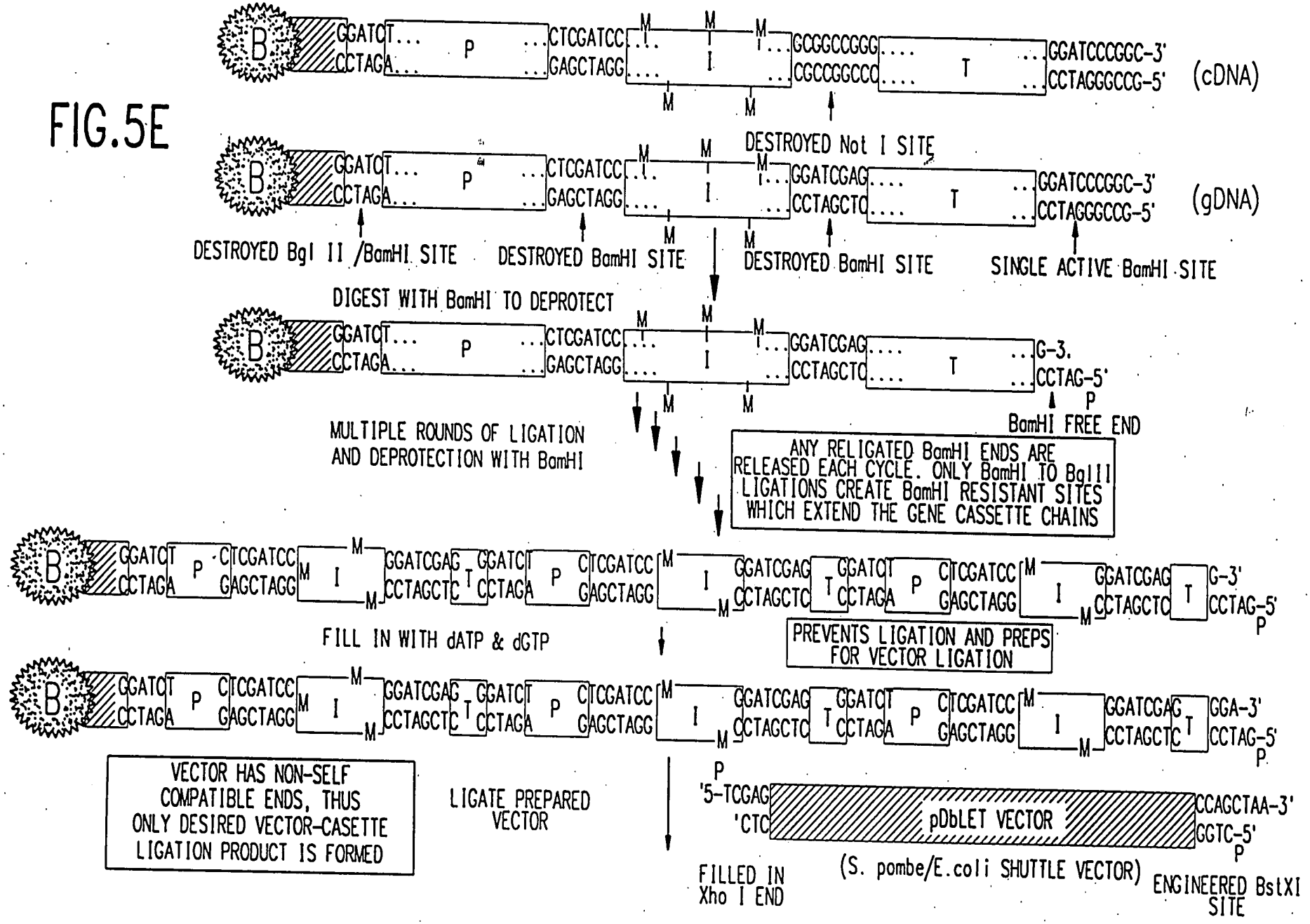

gDNA PARTIAL WITH Sau3AI
PROVIDES GENE FRAGMENTS
5' - GATCT
PCR PROMOTER
FRAGMENTS
CTC-3'
GAGCT-5'
Bgl II
Xho I
P
5'-GATCC
gDNA
INSERTS
G-3'
CCTAG-5'
3'-G
Sau3AI ENDS
P
5'-TCGAG
PCR TERMINATOR
FRAGMENTS
GGATCCCGGC-3'
CCTAGGGCCG-5'
Xho I
BamHI
METHYLATE WITH BamHI METHYLASE.
METHYLATION PROTECTS INTERNAL BamHI SITES FROM CLEAVAGE DURING DEPROTECTION STEP.
M
FILL IN WITH dATP & dGTP
GGA-3'
FILL IN PREVENTS SELF-LIGATION OF INSERTS & MAKES ENDS COMPATIBLE WITH PRO & TER PCR FRAGMENTS
SAMPLE cDNA GENE CASSETTES
CTCGATCC
GGATCGAG
PCR TERMINATOR FRAGMENT
PCR PROMOTER FRAGMENT
GAGCTAGG
CCTAGCTC
GENE CASSETTES READY TO LIGATE TO BEAD
B
BEAD W/INTRON NUCLEASE LINKER
BamHI END
P
I
T

FIG.5E


B
GGATCT
CCTAGA
P
CTCGATCC
GAGCTAGG
M
I
GCGGCCGGG
CGCCGGCCC
T
GGATCCCGGC-3'
CCTAGGGCCG-5'
(cDNA)
DESTROYED Not I SITE
GGATCGAG
CCTAGCTC
(gDNA)
DESTROYED Bgl II /BamHI SITE
DESTROYED BamHI SITE
DESTROYED BamHI SITE
SINGLE ACTIVE BamHI SITE
DIGEST WITH BamHI TO DEPROTECT
G-3.
CCTAG-5'
BamHI FREE END
MULTIPLE ROUNDS OF LIGATION
AND DEPROTECTION WITH BamHI
ANY RELIGATED BamHI ENDS ARE
RELEASED EACH CYCLE. ONLY BamHI TO BglII
LIGATIONS CREATE BamHI RESISTANT SITES
WHICH EXTEND THE GENE CASSETTE CHAINS
FILL IN WITH dATP & dGTP
PREVENTS LIGATION AND PREPS
FOR VECTOR LIGATION
GGA-3'
VECTOR HAS NON-SELF
COMPATIBLE ENDS, THUS
ONLY DESIRED VECTOR-CASETTE
LIGATION PRODUCT IS FORMED
LIGATE PREPARED
VECTOR
'5-TCGAG
'CTC
pDbLET VECTOR
CCAGCTAA-3'
GGTC-5'
FILLED IN
Xho I END
(S. pombe/E.coli SHUTTLE VECTOR)
ENGINEERED BstXI
SITE

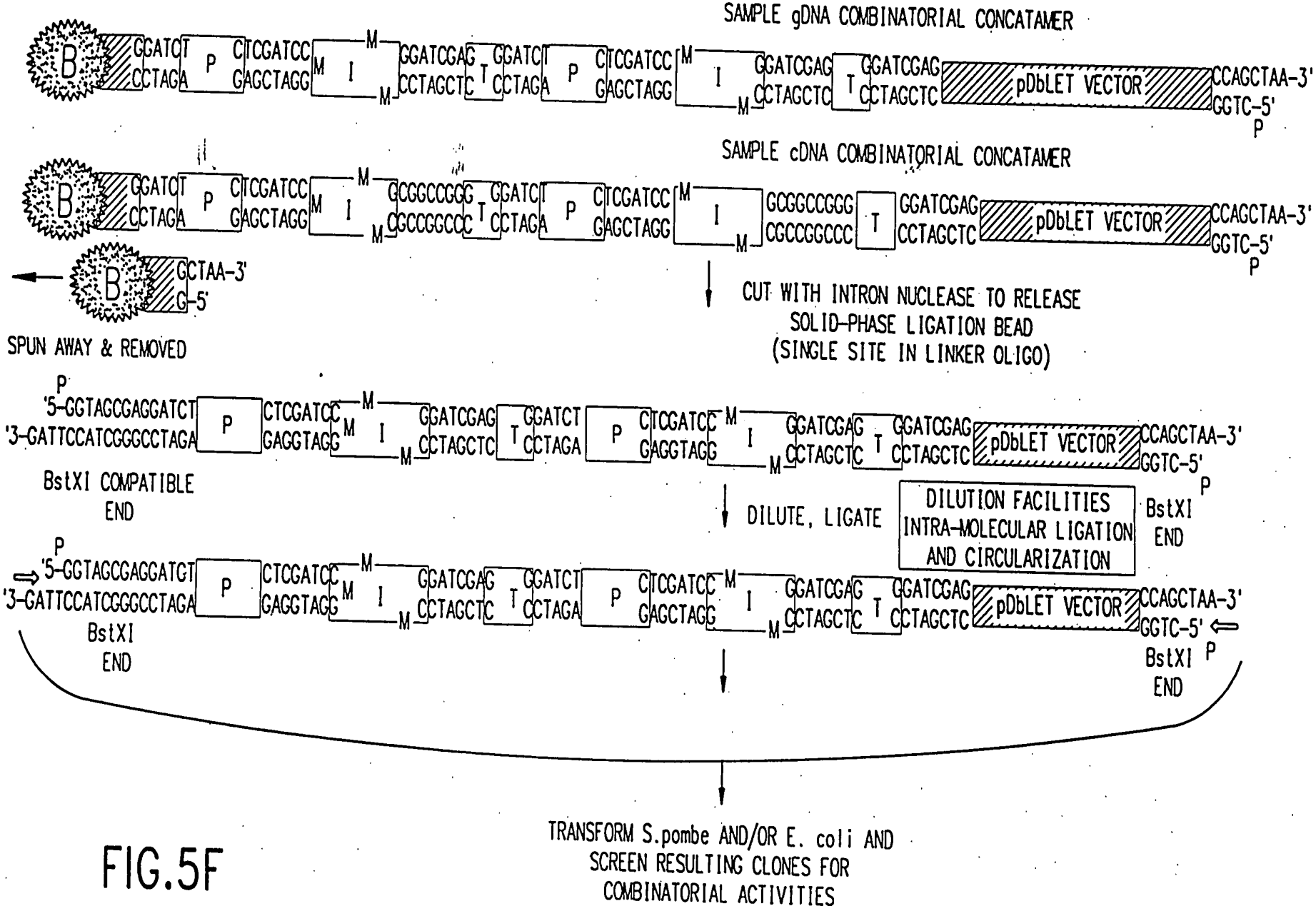

SAMPLE gDNA COMBINATORIAL CONCATAMER
SAMPLE cDNA COMBINATORIAL CONCATAMER
pDbLET VECTOR
SPUN AWAY & REMOVED
CUT WITH INTRON NUCLEASE TO RELEASE SOLID-PHASE LIGATION BEAD (SINGLE SITE IN LINKER OLIGO)
BstXI COMPATIBLE END
DILUTE, LIGATE
DILUTION FACILITIES INTRA-MOLECULAR LIGATION AND CIRCULARIZATION
BstXI END
TRANSFORM S.pombe AND/OR E. coli AND SCREEN RESULTING CLONES FOR COMBINATORIAL ACTIVITIES

FIG.5F

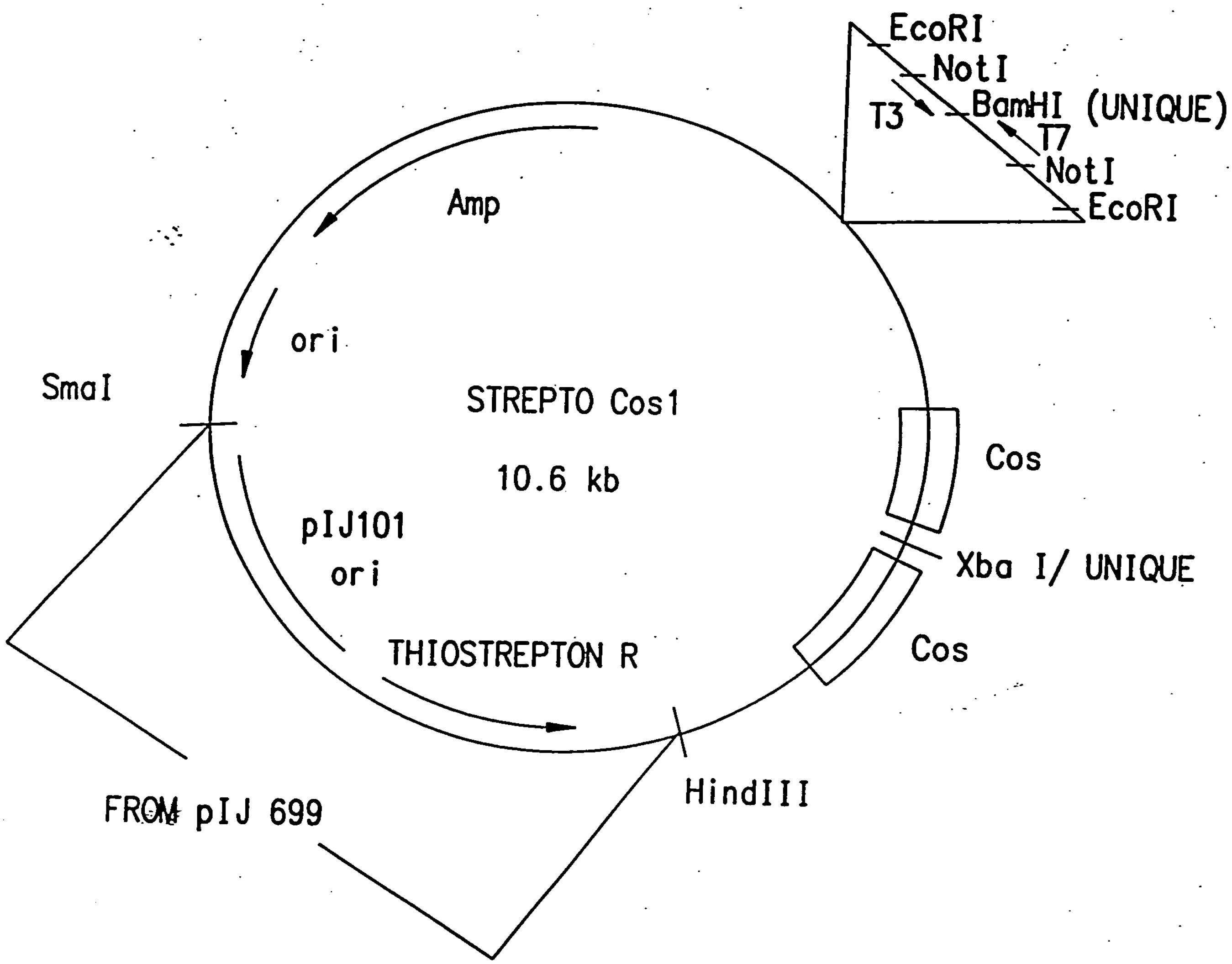
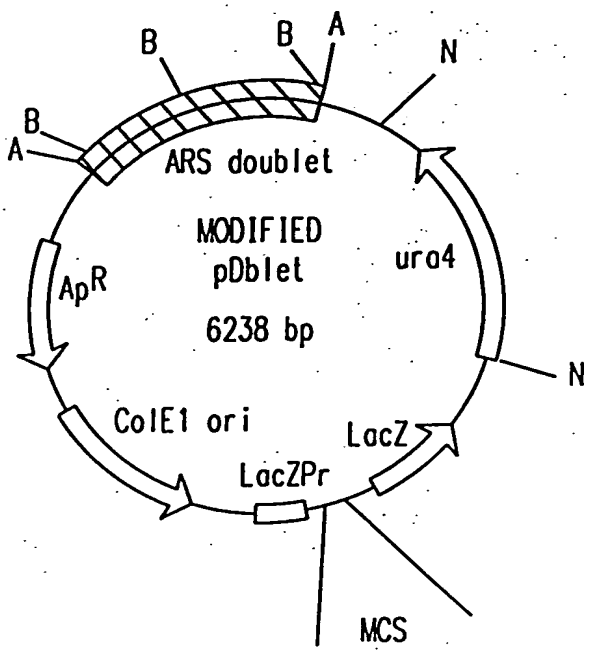

EcoRI
NotI
T3
BamHI (UNIQUE)
T7
NotI
EcoRI
Amp
ori
SmaI
STREPTO Cos1
10.6 kb
pIJ101
ori
THIOSTREPTON R
Cos
Xba I/ UNIQUE
Cos
HindIII
FROM pIJ 699

FIG.6A



MCS = SacI–NcoI–BstXI–NotI–Xba...

FIG.6B

5' CCTAGCCATGGCCACCTAACTGGGATCGC 3'

3' TCGAGGATCGGTACCGGTGGATTGACCCTAGCGCCGG 5'

SacI NcoI BstXI NotI END

FIG.6C

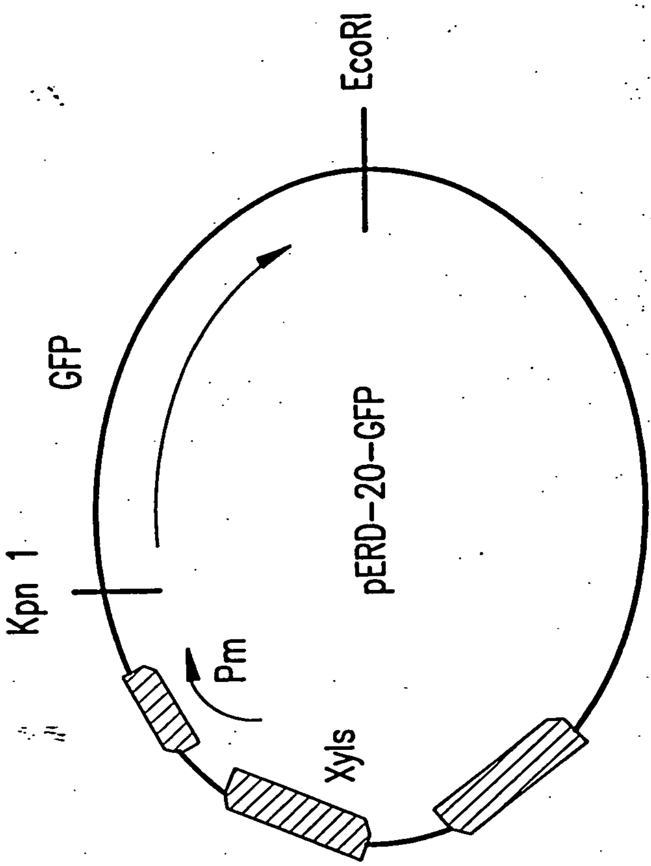


FIG.7

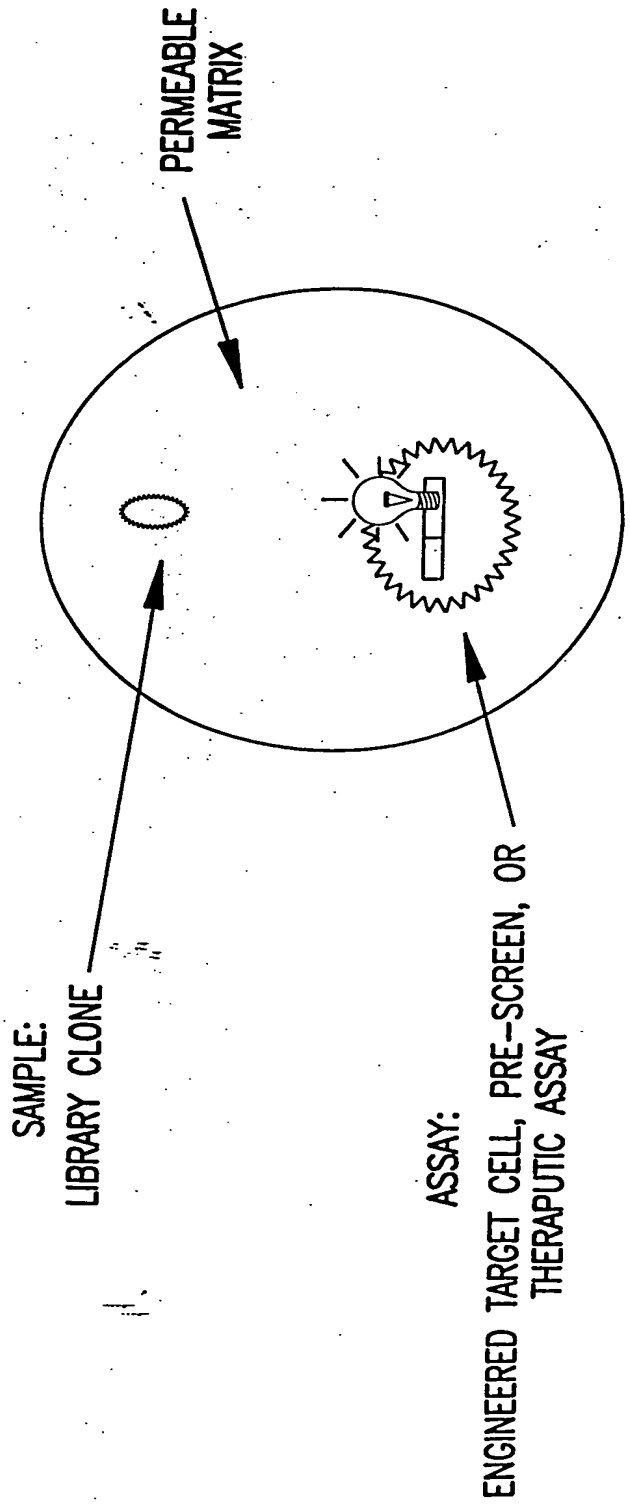

PERMEABLE MATRIX
SAMPLE: LIBRARY CLONE
ASSAY: ENGINEERED TARGET CELL, PRE-SCREEN, OR THERAPUTIC ASSAY
MACRODROPLET

FIG.8

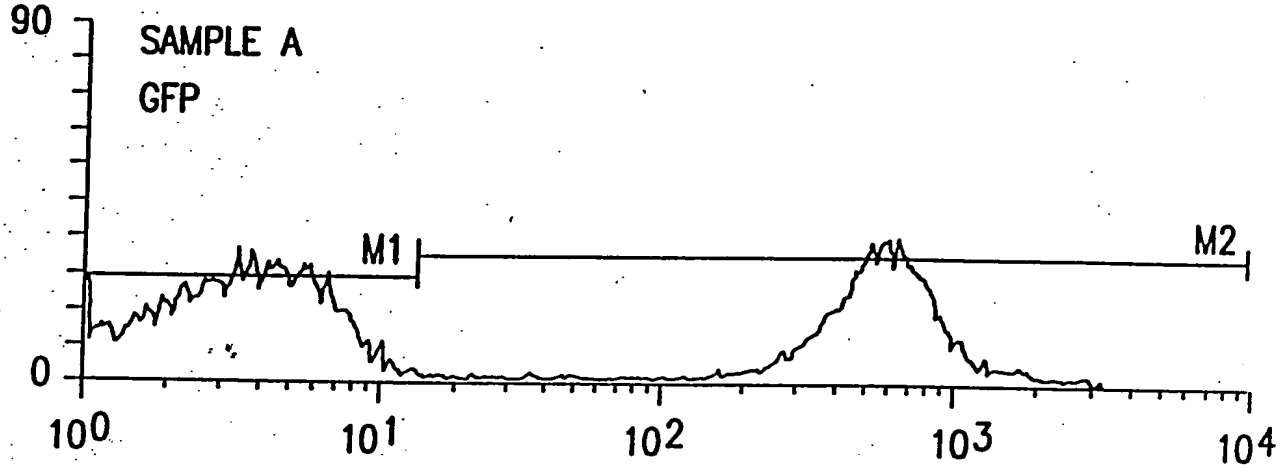


FIG.9A

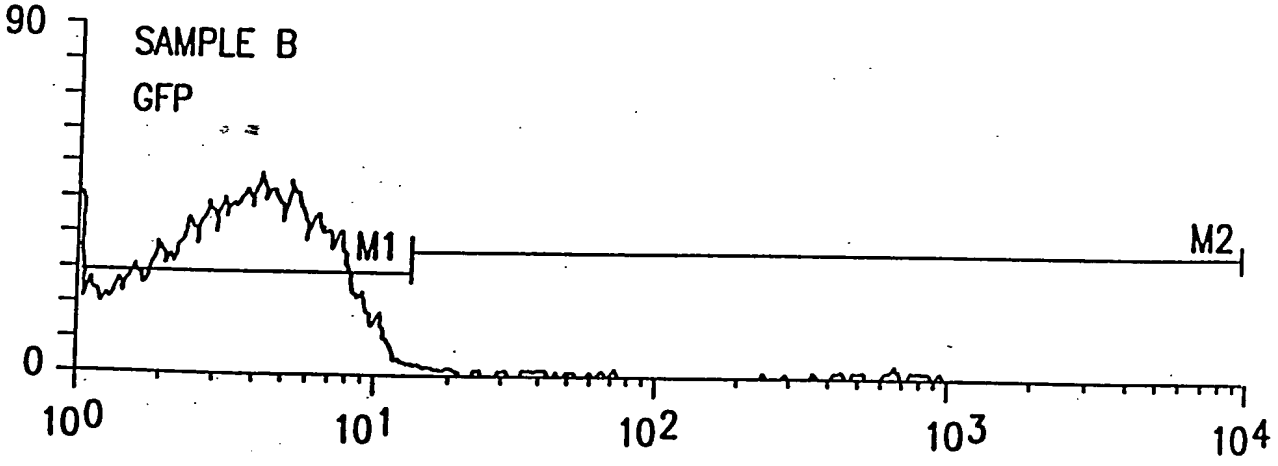


FIG.9B

```
                    POSITION
CXC-AMN20           (1) ----------------------------NSHFIITTQGVLVFDSGSSEHIGNA (23)
ACTINORHODIN DEHYDRASE   1 MTVEVREVAEGVYAYEQAPGGWCVSNAGIVVGGDGALVVDTLSTIPRARR  50

CXC-AMN20           (24) IIAAVKRVTEQPIRWVVNSHSHADHWLGNAALAKLGAELISTSLSAETMK (73)
ACTINORHODIN DEHYDRASE  51 LAEWVDKLAAGPGRTVVNTHFHGDHAFGNQVFAP-GTRIIAHEDMRSAMV  99

CXC-AMN20           (74) SDGPVDVKAFFNMTKGATGESTLVIPTSIILHQQTRTFGDTEVEFVFAND (123)
ACTINORHODIN DEHYDRASE 100 TTGLALTG-LWP--RVDWGEIELRPPNVTFRDRLTLHVGERQVELTCVGP 146

CXC-AMN20           (124) GHSPGDVMLWLPKQRILIGGDVVNSNFMPIMTPRGNITQLISVLKEVEQL (173)
ACTINORHODIN DEHYDRASE 147 AHTDHDVVVWLPEERVLFAGDVVMSGVTP-FALFGSVAGTLAALDRLAEL 195

CXC-AMN20           (174) SPLLVLTGHGENTSVKSVSRDIQFLTYASNAVHEALVKGTTPAKIQASLQ (223)
ACTINORHODIN DEHYDRASE 196 EPEVVVGGHGPVAGPEVIDANRDYLRWVQRLAADAVDRRLTPLQAARRAD 245

CXC-AMN20          (224) ATLRTKFGKAYQDFDTSISYLLEMMIDKQRLQFSPIT---------- (264)
ACTINORHODIN DEHYDRASE 246 LGAFAGLLDAERLVANLHRAHEELLGGHVRDAMEIFAELVAYNGGQLPTC 295

CXC-AMN20          -  --
ACTINORHODIN DEHYDRASE 296 LA*                                              297
```

FIG.10

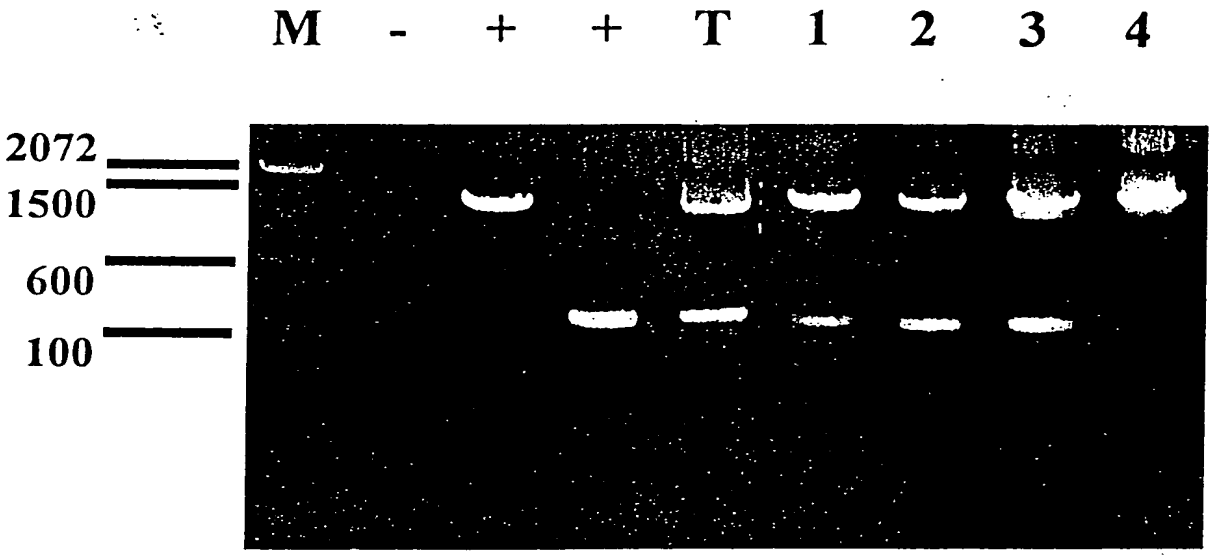


FIG. 11

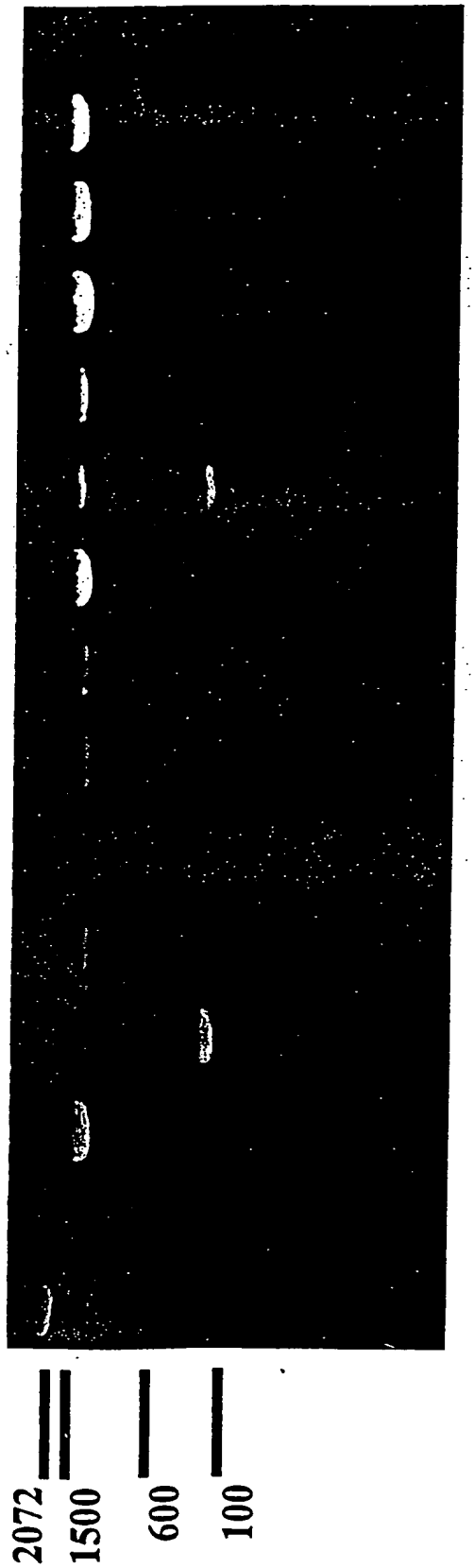


FIG.12A

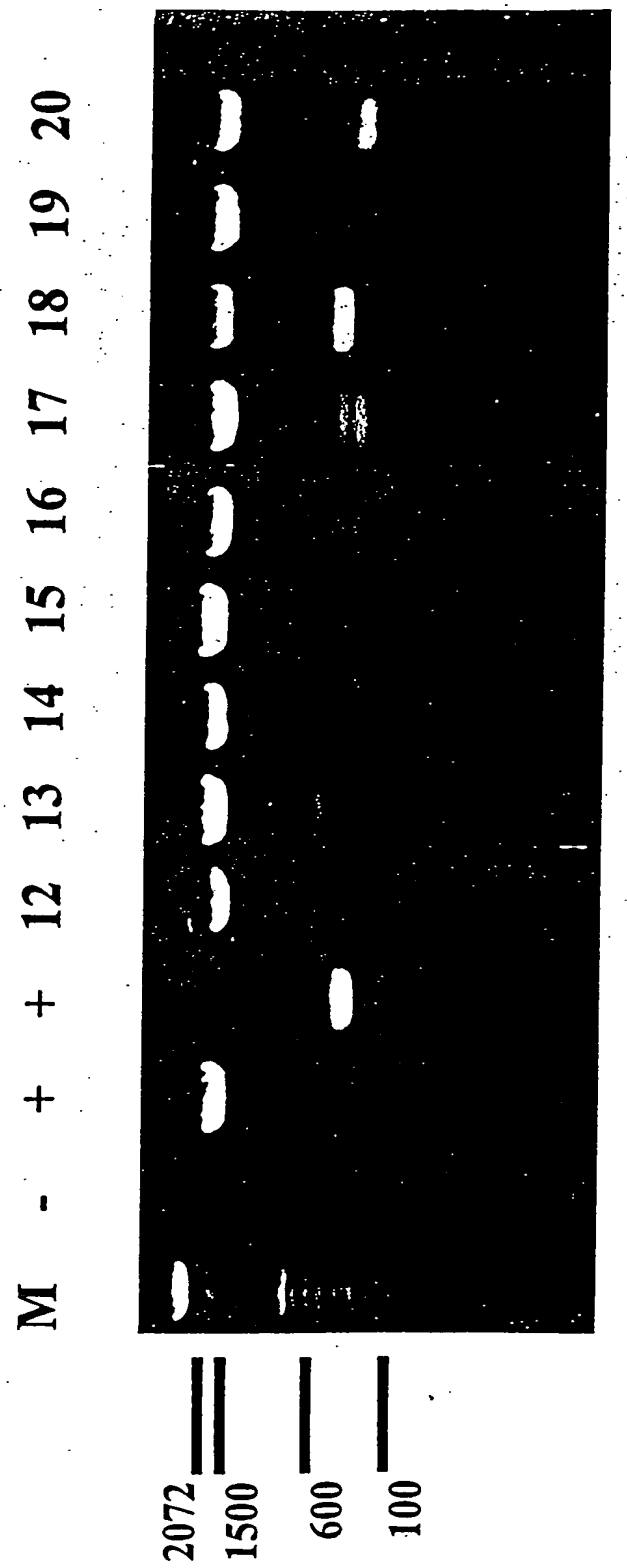


FIG.12B

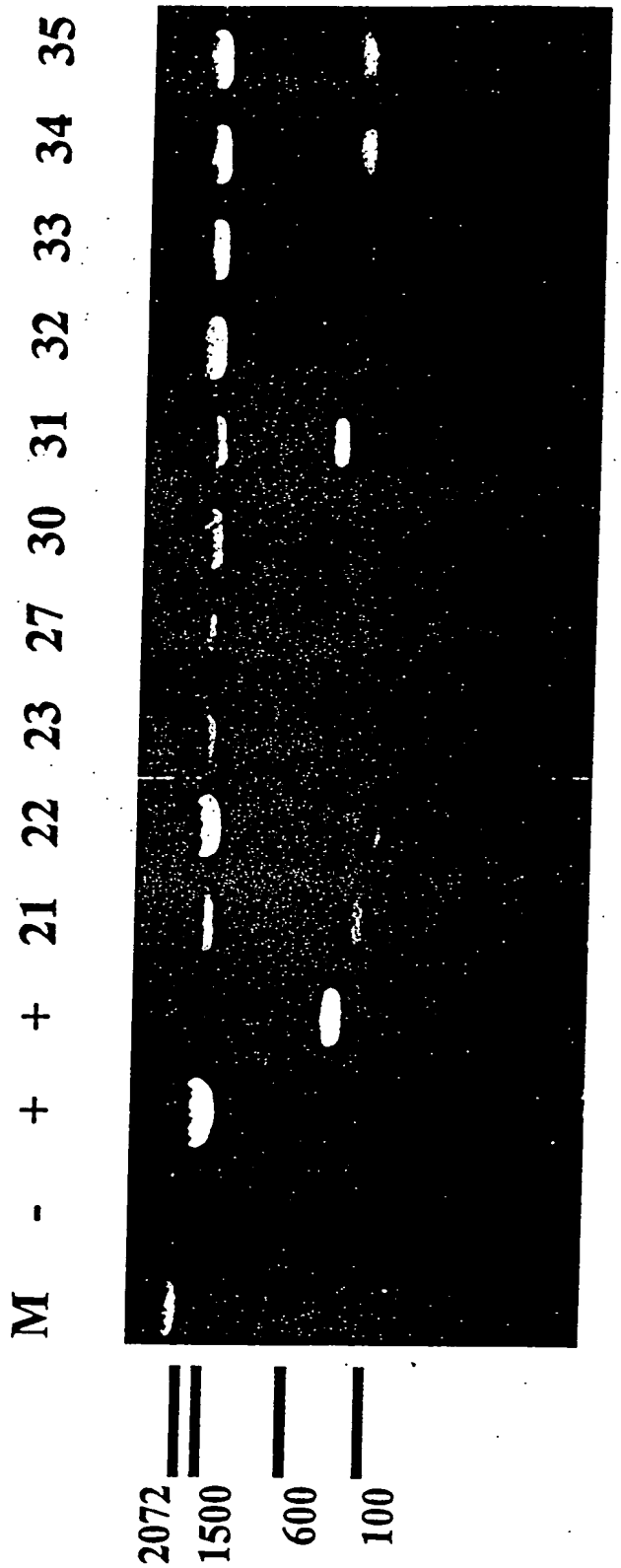


FIG.12C

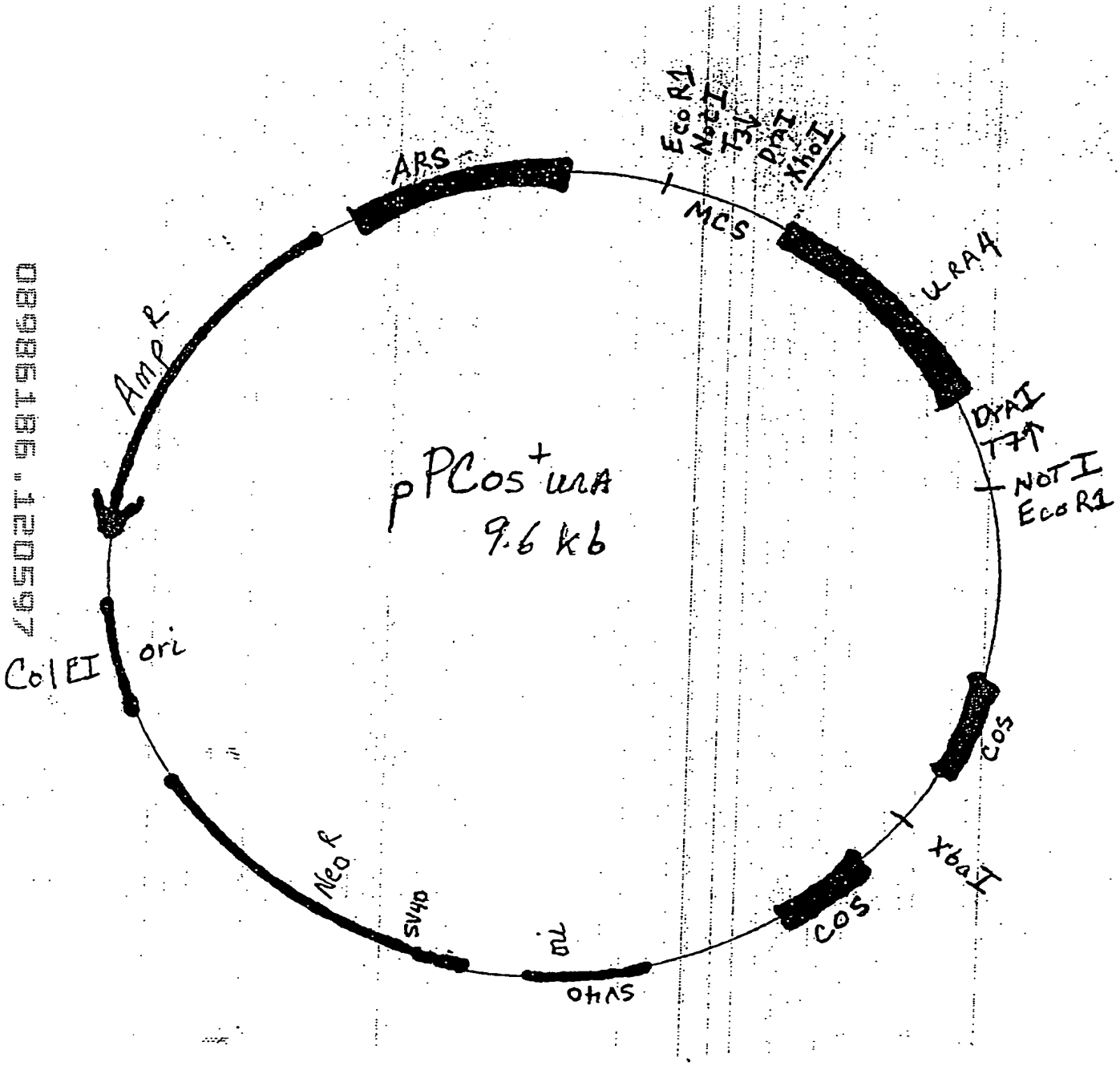

ARS
EcoR1
NotI
T3
DraI
XhoI
MCS
URA4
DraI
T7
NotI
EcoR1
COS
XbaI
COS
SV40
ori
Neo r
ColEI
Amp R
pPCos+ura
9.6 kb

FIG. 13

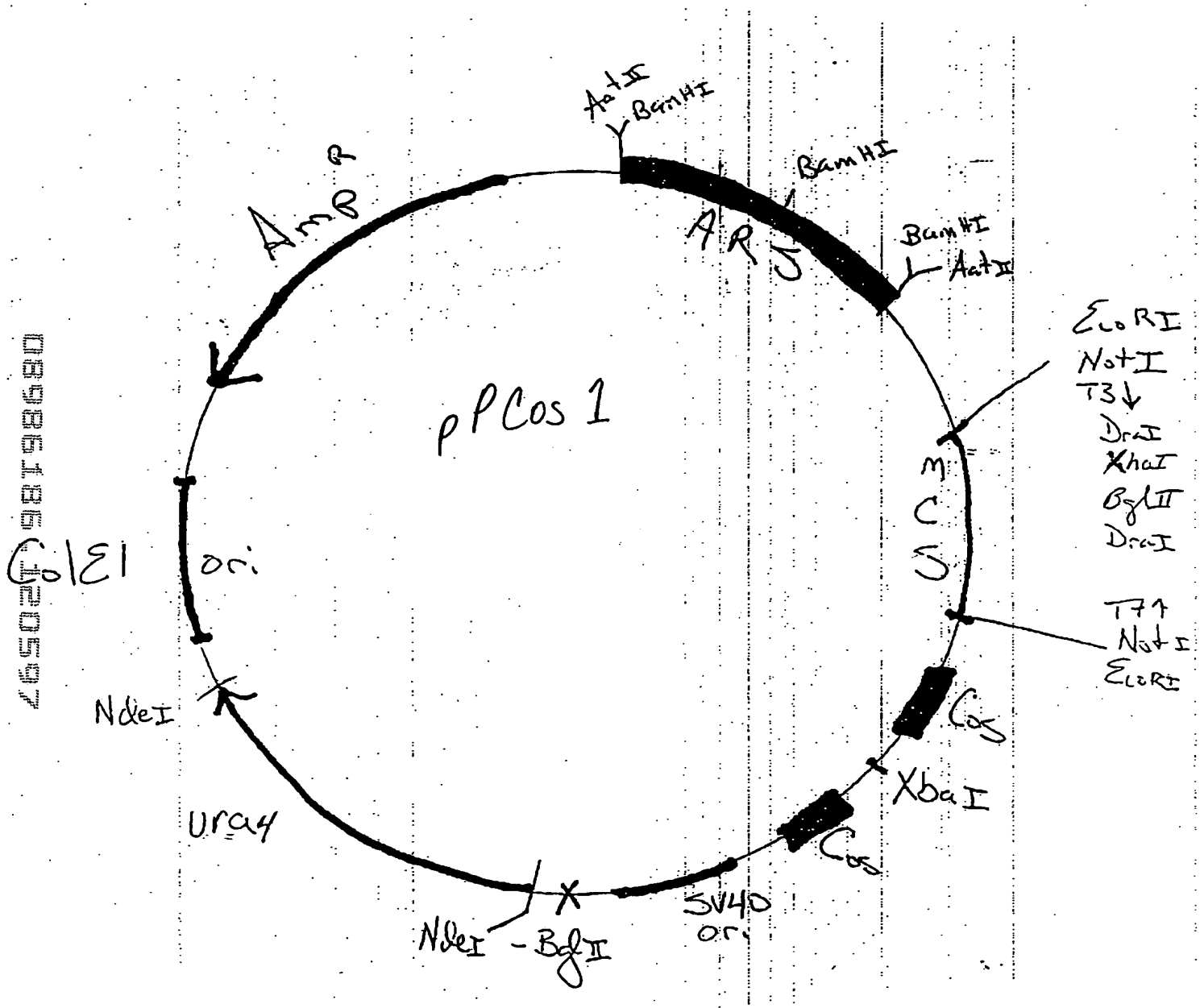

AatII
BamHI
BamHI
BamHI
AatII
AmpR
ARS
pPCos 1
EcoRI
NotI
T3
DraI
XhaI
BglII
DraI
MCS
T7
NotI
EcoRI
Cos
XbaI
Cos
SV40 ori
NdeI - BglII
Ura4
NdeI
ColE1 ori

FIG. 14